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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/583,277	06/16/2006	Yoshiko Minakuchi	0020-5493PUS1	2615

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EXAMINER	
NGUYEN, QUANG	
ART UNIT	PAPER NUMBER
1633	

NOTIFICATION DATE	DELIVERY MODE
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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

mailroom@bskb.com

Office Action Summary	Application No.	Applicant(s)	
	10/583,277	MINAKUCHI ET AL.	
	Examiner	Art Unit	
	QUANG NGUYEN, Ph.D.	1633	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *13 April 2009*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-14 and 16-21* is/are pending in the application.

4a) Of the above claim(s) *4,14 and 16-21* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-3 and 5-13* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *16 June 2006* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☒ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☒ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *6/16/06;12/22/06;10/31/07*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Claims 1-14 and 16-21 are pending in the present application.

Applicant's election of Group I (claims 1-13) with traverse in the reply filed on 4/13/09 is acknowledged. Additionally, Applicants elected siRNA as a species of a nucleic acid. Because applicant did not distinctly and specifically point out the supposed errors in the restriction requirement, the election has been treated as an election without traverse (MPEP § 818.03(a)).

Accordingly, claims 14 and 16-21 were withdrawn from further consideration because they are directed to a non-elected invention. Additionally, claim 4 was also withdrawn from further consideration because it is directed to a non-elected species.

Therefore, claims 1-3 and 5-13 are examined on the merits herein with the above elected species.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1-3 and 5-13 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for:

A method of nucleic acid transfer comprising the following steps (a) and (b):

(a) contacting a nucleic acid with a cell in a medium; and

(b) following the step (a), contacting the medium of (a) with **a volume of a high concentration solution of calcium chloride or calcium phosphate, wherein the concentration of calcium chloride or calcium phosphate in the medium of step (b) is up to 30.1 mM**;

does not reasonably provide enablement for **a method of nucleic acid transfer using any amount of a high-concentration solution of any other metal salts as broadly claimed**. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention commensurate in scope with these claims.

The present disclosure is not enabled for the instant broadly claimed invention for the reasons discussed below.

1. The breadth of the claims

The instant claims are directed to a method of nucleic acid transfer comprising the steps of: (a) contacting any nucleic acid (with siRNA as the elected species) in any form (e.g., in a complex, in an inclusion body or in a living body-derived substance) with a cell in a medium; and (b) following the step (a), contacting the medium of (a) with any amount of a high-concentration solution of any metal salt, including calcium chloride. It is noted that as defined by the instant specification (page 13, lines 16-20), the term "high –concentration" refers to any concentration of 0.1M or greater (e.g., 1M, 2M, 3M, 5M, 10M…).

2. The state and the unpredictability of the prior art

At about the effective filing date of the present application (12/19/2003), little was known about the effect of the addition of high-concentration of any metal salt into a medium of a cell culture following the step of contacting a nucleic acid with a cell as evidenced at least by the teachings of Rocha et al. (J. Physiol. Biochem. 58:45-56, 2002; IDS), Haberland et al. (BBA 1445:21-30, 1999), Haberland et al. (Pharmaceutical Res. 17:229-235, 2000) and Lam et al (BBA 1463 :279-290, 2000). It was known, however, that the addition of soluble calcium ions (2 mM) or calcium phosphate precipitates to cells after a transfection period enhances transfection of polycation-mediated non-viral DNA transfer systems; calcium ion is not needed for non-viral DNA complex uptake; **and the presence of calcium ions and not magnesium ions in the post-incubation medium** after transfection can overcome serum inhibition as taught by Haberland et al. (BBA 1445:21-30, 1999) and Haberland et al. (Pharmaceutical Res. 17:229-235, 2000). Moreover, Lam et al taught that the enhanced *in vitro* transfection potency of plasmid DNA-cationic liposome complexes **is specific for calcium ions, and not for other cations such as Mg or Na ions**, and that cellular cytotoxicity was observed for calcium concentration at 50 mM or greater when used for forming DNA complexes (see at least the abstract and page 282, col. 1, last paragraph).

Furthermore, the physiological art is recognized as unpredictable (MPEP 2164.03).

3. The amount of direction or guidance provided

Apart from disclosing the addition of calcium chloride to a medium of a cell up to 30.1 mM, following the step of contacting the cell with a nucleic acid molecule resulted

in an efficient gene expression, the instant specification fails to provide sufficient guidance for a skilled artisan on how to attain a similar effect using any other metal salts or at any other concentrations higher than the disclosed 30.1 mM as broadly claimed. Since a particular transfection effect can be attributed to a specific metal salt and not necessarily extended to others as taught in the prior art as discussed above, coupled with the lack of sufficient guidance provided by the instant specification it would have required undue experimentation for a skilled artisan to make and/or use a method of nucleic acid transfer as broadly claimed.

As set forth in *In re Fisher*, 166 USPQ 18 (CCPA 1970), compliance with 35 USC 112, first paragraph requires:

> That scope of claims must bear a reasonable correlation to scope of enablement provided by specification to persons of ordinary skill in the are; in cases involving predictable factors, such as mechanical or electrical elements, a single embodiment provides broad enablement in the sense that, once imagined, other embodiments can be made without difficulty and their performance characteristics predicted by resort to known scientific laws; in cases involving unpredictable factors, such as most chemical reactions and physiological activity, scope of enablement varies inversely with degree of unpredictability of factors involved.

Accordingly, due to the lack of sufficient guidance provided by the specification regarding to the issues set forth above, the breadth of the claims, and the state and the unpredictability of the relevant art, it would have required undue experimentation for one skilled in the art to make and use the instant broadly claimed invention.

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 5 and 10-11 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

A broad range or limitation together with a narrow range or limitation that falls within the broad range or limitation (in the same claim) is considered indefinite, since the resulting claim does not clearly set forth the metes and bounds of the patent protection desired. See MPEP § 2173.05(c). Note the explanation given by the Board of Patent Appeals and Interferences in *Ex parte Wu*, 10 USPQ2d 2031, 2033 (Bd. Pat. App. & Inter. 1989), as to where broad language is followed by "such as" and then narrow language. The Board stated that this can render a claim indefinite by raising a question or doubt as to whether the feature introduced by such language is (a) merely exemplary of the remainder of the claim, and therefore not required, or (b) a required feature of the claims. Note also, for example, the decisions of *Ex parte Steigewald*, 131 USPQ 74 (Bd. App. 1961); *Ex parte Hall*, 83 USPQ 38 (Bd. App. 1948); and *Ex parte Hasche*, 86 USPQ 481 (Bd. App. 1949). In the present instance, claim 5 recites the broad recitation “cross-linked nucleic acid”, and the claim also recites “(locked nucleic acid; LNA) which is the narrower statement of the range/limitation. Please note that not every cross-linked nucleic acid is also a locked nucleic acid or LNA as described by Singh et al., Chem. Commun. 455, 1998 as referenced on page 10, lines 19-20 of the instant specification.

Claims 10 and 11 recite the limitation "**the volume**" in line 2 of the claim 10. There is insufficient antecedent basis for this limitation in the claim because in claim 1

from which both claims 10 and 11 are dependent on, there is no recitation of any volume.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 1-3, 5-6 and 8-13 are rejected under 35 U.S.C. 103(a) as being unpatentable over Li et al (US 2004/0147475) in view of Haberland et al. (BBA 1445:21-30, 1999), Haberland et al. (Pharmaceutical Res. 17:229-235, 2000), and Chappel, S.C. (EP 0779362; IDS).

With respect to the scope of enablement and the elected species, Li et al disclose a method for introducing dsRNAs or siRNAs into cells, cell culture, organs and

tissues, and whole organisms to attenuate gene expression (see at least the abstract; Summary of the invention, particularly paragraphs 35-54 and claims). Li et al teach specifically that the dsRNA nucleotide sequence is preferably at least about 25 bases and that it can be introudced into a cell in various ways, including liposome-mediated delivery, viral infection, transformation, transfection mediated by calcium phosphate, electroporation among others (paragraphs 39 and 44). In an exemplification Li et al disclose that rat cells were transfected by **overlaying onto the cells with lipid-DNA complexes containing dsGFP RNA in serum-free DMEM and incubated for 5 hours at 37 ^{0}C, following by the addition of DMEM (1 mL) with 20% FBS without removing the transfection mixture** (see example III on page 12).

Li et al do not teach a cell culture method in which following the step of contacting a nucleic acid with a cell in a medium, further contacting the medium with a volume of a high-concentration solution of a calcium chloride.

However, at the effective filing date of the present application, Haberland et al (BBA 1445:21-30, 1999) already disclosed that **the addition of soluble calcium ions (2 mM) or calcium phosphate precipitates to cells after a transfection period enhances transfection of polycation-mediated non-viral DNA transfer systems, and that calcium ion is not needed for non-viral DNA complex uptake** (see at least the abstract; and particularly Figure 3).

Additionally, Haberland et al. (Pharmaceutical Res. 17:229-235, 2000) also demonstrated **the importance of the presence of calcium ions in the post-incubation medium after transfection for overcoming serum inhibition in a**

polycationic or cationic liposomal gene transfer system (see at least the abstract and Figures 3-4 and 7).

Furthermore, Chappel also disclosed **the use of an aliquot (31 ul) of a high-concentration of a calcium chloride solution (2 M) for the preparation of a DNA-calcium phosphate sample for cell transfection** (see page 11, section entitled "Calcium phosphate transfection").

Accordingly, it would have been obvious and within the scope of skill for an ordinary artisan to modify the teachings of Li et al at least with respect to a method for introducing dsRNAs or siRNAs into cells in a cell culture to attenuate gene expression by adding an appropriate volume of a high-concentration calcium chloride and/or calcium phosphate solution (concentration of 0.1M or greater; see definition of the term "high-concentration" on page 13, lines 16-20 of the instant specification) to the medium to attain at least a final concentration of calcium chloride or calcium phosphate of 2 mM following the step of contacting a nucleic acid with a cell in light of the teachings of Haberland et al (BBA 1445:21-30, 1999), Haberland et al. (Pharmaceutical Res. 17:229-235, 2000) and Chappel as presented above. With respect to the limitation of dependent claims 10-11, it would also have been obvious for an ordinary skilled artisan to use an aliquot within the range of 1 uL-20 uL of a high-concentration calcium chloride or calcium phosphate solution per 500 uL of the medium to attain the desired final calcium chloride or calcium phosphate concentration.

An ordinary skilled artisan would have been motivated to carry out the above modifications because both Haberland et al references demonstrated that the addition

of soluble calcium ions (2 mM) or calcium phosphate precipitates to cells **after a transfection period enhances transfection of polycation-mediated non-viral DNA transfer systems; calcium ion is not needed for non-viral DNA complex uptake; and the presence of calcium ions in the post-incubation medium after transfection can overcome serum inhibition.** Haberland et al. (Pharmaceutical Res. 17:229-235, 2000) also noted that gene therapy requires the transfectant-DNA complex to be resistant to serum as well as blood. Furthermore, the use of an appropriate aliquot of a high-concentration of calcium chloride solution such as the 2M $CaCl_2$ solution in a transfection protocol has also been taught by Chappel.

An ordinary skilled artisan would have a reasonable expectation of success to carry out the above modification in light of the teachings of Li et al., Haberland et al. (BBA 1445:21-30, 1999), Haberland et al. (Pharmaceutical Res. 17:229-235, 2000), and Chappel, S.C. ; coupled with a high level of skills of an ordinary skilled artisan in the relevant art.

Therefore, the claimed invention as a whole was *prima facie* obvious in the absence of evidence to the contrary.

Claim 7 is rejected under 35 U.S.C. 103(a) as being unpatentable over over Li et al (US 2004/0147475) in view of Haberland et al. (BBA 1445:21-30, 1999), Haberland et al. (Pharmaceutical Res. 17:229-235, 2000), and Chappel, S.C. (EP 0779362; IDS) as applied to claims 1-3, 5-6 and 8-13 above, and further in view of Kubota et al. (US 2004/0052840; IDS).

The combined teachings of Li et al, of Haberland et al. (BBA 1445:21-30, 1999), Haberland et al. (Pharmaceutical Res. 17:229-235, 2000) and Chappel were presented above. However, none of the cited references teaches specifically that the nucleic acid is in a form with atelocollagen.

At the effective filing date of the present application, Kubota et al already taught **an efficient preparation for transferring anti-sense oligonucleotides into a target cell that contains a collagen or atelocollagen as an essential component** (see at least the abstract; paragraphs 95, 101-104 and 108-112).

Accordingly, it would have been obvious and within the scope of skill for an ordinary artisan to further modify the combined teachings of Li et al, of Haberland et al. (BBA 1445:21-30, 1999), Haberland et al. (Pharmaceutical Res. 17:229-235, 2000) and Chappel set forth above by also utilizing a formulation of a nucleic acid (e.g., dsRNA or siRNA) containing atelocollagen for attenuating a target gene expression in cells of a cell culture in light of the teachings of Kubota et al.

An ordinary skilled artisan would have been further motivated to carry out the above modification because Kubota et al already taught that the preparation of an anti-sense oligonucleotide containing a collagen or atelocollagen was found to be efficient for transferring anti-sense oligonucleotides into a target cell due to its intrinsic advantages cited in paragraph 112.

An ordinary skilled artisan would have a reasonable expectation of success to carry out the above modification in light of the teachings of Li et al., Haberland et al. (BBA 1445:21-30, 1999), Haberland et al. (Pharmaceutical Res. 17:229-235, 2000),

Chappel, S.C. and Kubota et al.; coupled with a high level of skills of an ordinary skilled artisan in the relevant art.

Therefore, the claimed invention as a whole was *prima facie* obvious in the absence of evidence to the contrary.

Conclusion

No claim is allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Quang Nguyen, Ph.D., whose telephone number is (571) 272-0776.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's SPE, Joseph T. Woitach, Ph.D., may be reached at (571) 272-0739.

To aid in correlating any papers for this application, all further correspondence regarding this application should be directed to Group Art Unit 1633; Central Fax No. (571) 273-8300.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to (571) 272-0547.

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/QUANG NGUYEN/
Primary Examiner, Art Unit 1633